Biosensing with a Graphene-Based FET

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Introduction: A low-cost sensor that continuously and rapidly looks for a range of biomolecules is a longstanding goal for the biosensor community that remains unfulfilled. Such real-time sensors could lead to "more effective point of injury care for Sailors and Marines," and "enhanced health and warfighter performance both afloat and ashore."[1]

The arduous quest for such an "ultimate" biosensor, one that fully meets the Office of Naval Research (ONR) vision of enhancing warfighter health and survivability, has spanned nearly 50 years. One particular application of such a sensor, medical diagnostics, utilizes biosensor technologies based on nucleic acid and immunoassays. Most of these biosensor technologies currently use a label to facilitate detection. A label can be a fluorescent molecule or a magnetic bead or any component that helps signal the presence of the target biomolecule. In particular, fluorescence labels remain the predominant strategy because of their signal strength, availability of multiple emission wavelengths, and their compatibility with multiplexed, high-throughput, *in situ* and *in vivo* applications. The difficulty is that labels introduce complexity into the sensor system and, in the case of fluorescence labels, are consumed, whereby detection can only last a defined duration. In contrast, "label-free sensing" eliminates sample handling to attach the label to the target and alleviates steric hindrance, thus resulting in higher sensitivity, faster response, and ultimately lower costs.

A popular approach for label-free sensing is based on the field effect transistor (FET), which consists of two terminals, the source and drain, and a gate that controls the resistance of the device (Fig. 1, top). For sensor applications, the gate terminal is replaced by a material that can sense biomolecules. Typically, it consists of covalently bound "probe" biomolecules that can capture the target biomolecule via biological interactions such as protein–ligand and DNA hybridization. We refer to these modified structures as biologically active field effect transistors, or BioFETs (Fig. 1, bottom). Several promising biosensing advances have used nanoscale one-dimensional materials such as carbon nanotubes as the gate.[2] Recently, we demonstrated that an ideal gate material for BioFETs is chemically modified graphene (CMG).[3] Because graphene is only one atom thick, the highly mobile electrons at or near its surface are extremely sensitive to local charge changes. Because most biomolecules are charged, their binding to a graphene-based gate will disrupt the flow of the electrons. Of course, this only works if the graphene is properly biofunctionalized such that protein–ligand interactions or nucleic acid hybridization can occur.

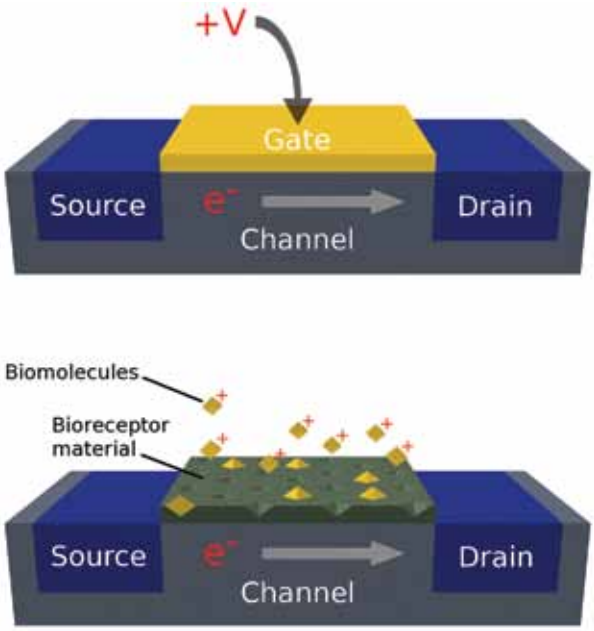

FIGURE 1
BioFET concept. The gate electrode of a standard FET (top) is replaced by biological components (bottom). Changes in charge distribution at the gate, due to biomolecular recognition activity, modulate current transport through the semiconductor.

Graphene Biofunctionalization: Unfortunately, pristine graphene is inert, and chemical sites to which biomolecules can be easily covalently tethered must be carefully created. We have pursued two independent pathways: (1) the modification of reduced graphene oxide (rGO), and (2) the implantation of chemical attachment sites into chemical vapor deposition (CVD)-grown graphene sheets via ultra-low-temperature gas plasmas.

In the first pathway, graphite powder is converted to graphene oxide (GO) using strong oxidizers and sonication to produce a material rich with epoxyl, carboxyl, and hydroxyl groups, which are ideal for biofunctionalization. Unlike graphene, however, GO is electrically insulating, and the traditional method of using hydrazine reduction to restore conductivity strips the GO of these facile attachment points. Faced with the contradictory requirements to retain the oxide functional groups for biofunctionalization

and to remove them to regain conductivity, we found a breakthrough in the form of selective reduction of the GO material.[3] A placeholder molecule, the bifunctional cross-linker ethylenediamine (EDA), is reacted with GO's epoxide groups prior to reduction. The entire film is then reduced and the bound EDA survives the aggressive reduction process to leave behind a primary amine group, an ideal attachment site for coupling to DNA or proteins. This was the critical step because it resulted in a CMG that was both highly conductive and supported covalent attachment of biomolecules.

In the second pathway, graphene was produced by low-pressure (1 Torr) CVD on copper foils by the NRL Electronic Materials Branch and then transferred from the copper foil to silicon/SiO_2 substrates via wet etching. The material was then treated using an ammonia (NH_3)-containing plasma process developed in the NRL Plasma Physics Division.[4] As in the previous pathway, the end result is incorporation of primary amines in the CVD graphene sheet that can bind to DNA and proteins. The appeal of this second pathway is straightforward: the direct and controllable addition of chemical attachment points to pristine graphene. Such control over attachment point density has been shown to be critical in past efforts at developing biosensor chemistry. This is in sharp contrast to the first approach, which uses nonconductive GO, which, while cheaper to produce, has an abundance of defects that necessitate reduction to regain the conductivity required for biosensing.

Sensor Construction and Measurement: Graphene sheets are transferred over pre-patterned interdigitated gold electrodes on SiO_2 substrates. The sensing areas encompassing the electrodes are defined by photolithography, and excess graphene is removed via oxygen plasma etch. The non-sensing areas of the gold electrodes are protected by a layer of either Si_3N_4 or Al_2O_3. For the rGO BioFET, single-stranded DNA probes are covalently attached via the EDA pathway described above. For the CVD-grown graphene BioFET, primary amines are introduced by exposure to a low-pressure (75 mTorr) Ar/NH_3 plasma. For both types of sensors, primary amine terminated DNA is linked via glutaraldehyde to create probe spots. In each experiment, two BioFETs are operated simultaneously using a lock-in amplifier in a differential measurement scheme. One BioFET contains single-stranded DNA probes for a complementary single-stranded DNA target. The other BioFET acts as a reference with probes for a noncomplementary DNA target. Taking the difference of the signals from these two sensors results in specific binding signal only.

Performance: Figure 2 is a performance comparison of the BioFET sensors constructed by the two described methods. The sensors were exposed to 1 μM concentration of target and control DNA in a 15 mM phosphate-buffered saline solution. As can be seen, the CVD graphene functionalized with primary amines via controlled plasma exposure shows a 4 times larger signal-to-noise ratio compared to our rGO sensors. In addition, we showed the dose-dependent response on a set of rGO BioFET sensors to changes in complementary DNA concentrations between 1 μM and 10 nM with a projected 2 nM detection limit (Fig. 3).[3] More recently, improvements in electronic noise levels and rGO surface biofunctionalization chemistries have resulted in preliminary data indicating high pM sensitivity (unpublished data) — a result that begins to challenge traditional analytical techniques.

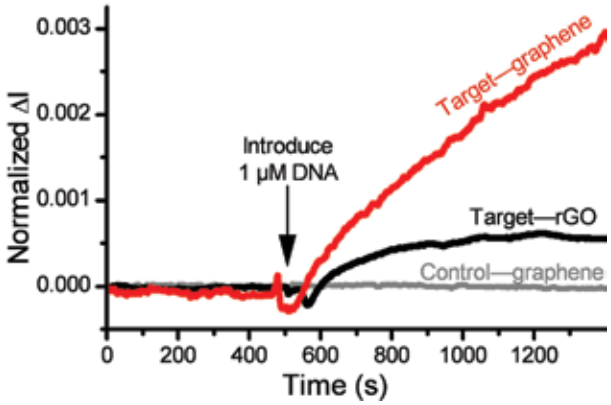

FIGURE 2
Response of aminated graphene DNA sensor to both target and control solutions. Target is complementary to probe; control is noncomplementary to probe. The sensing response is compared to that seen from a reduced graphene oxide (rGO) DNA sensor for an identical target solution. It shows an enhancement of the signal-to-noise ratio as compared to the rGO sensor.

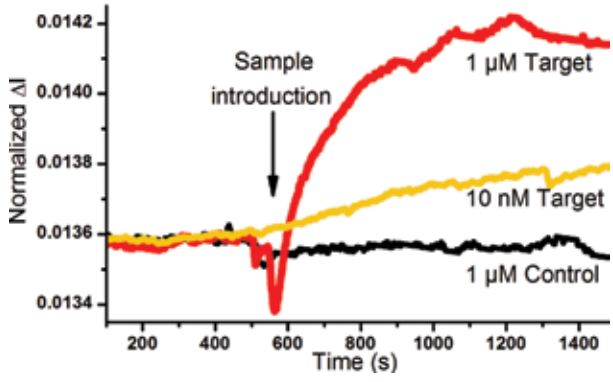

FIGURE 3
Unoptimized rGO-based dual BioFET DNA detection. Target is complementary to probe on biofunctionalized rGO; control is noncomplementary to probe. Signal is the difference between the reference BioFET and the DNA-functionalized BioFET.

Summary: We have developed chemical modification procedures on graphene for the attachment of biorecognition molecules (e.g., antibodies and DNA),

to achieve specific target binding and a signal response without the need for a label (e.g., optical or magnetic). The high electron mobility near or at the surface of graphene-based BioFETs gives these devices their high sensitivity. We have shown that modifying the graphene surfaces to accept biologically sensitive material with native charges that disrupt the flow of the FET's electrons is a winning strategy that has enormous potential for satisfying many of the criteria for an ideal biosensor: rapid, sensitive, label-free detection in an inexpensive, compact, low-power, electronically integrated module. The low inherent cost of these systems makes them especially appealing. Both CVD graphene and rGO are inexpensive to produce and can be transferred to low-cost flexible substrates such as plastics. Current efforts aim to produce such devices and to minimize steps requiring a clean room by using, for instance, ink jet printing to deposit electrodes and isolation layers.

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Silicon Spintronics at 500 K

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Silicon Spintronics: The control of spin-polarized carrier populations and currents offers a new paradigm for information processing in semiconductors. The *International Technology Roadmap for Semiconductors*[1] has identified the electron's spin as a new state variable, a "spintronic" approach, to be explored beyond Moore's Law. Silicon (Si) is an ideal host for such a spin-based technology, because its low atomic mass and crystal inversion symmetry result in very small spin orbit interactions and long spin lifetimes.

Challenge: Although the steady reduction in device feature size predicted by Moore's Law has been remarkably successful, critical device dimensions now approach atomic length scales, so that further size scaling becomes untenable. Researchers have been forced to look beyond the simple reduction of size to develop future generations of electronic devices. Utilization of the electron's spin angular momentum as an alternate state variable provides additional functionality and performance for future electronic devices envisioned beyond the current roadmap. Electrical generation, manipulation, and detection of significant spin polarization in silicon at temperatures that meet commercial and military requirements are essential to validate spin as an alternative to charge for a device technology beyond Moore's Law.

Spin Accumulation in Si to 500 K: To make a semiconductor spintronic device, one needs contacts that can both generate a current of spin-polarized electrons (spin injector), and detect the spin polarization of the electrons (spin detector) in the semiconductor.[2] A material with a spin-polarized density of states such as a ferromagnetic (FM) metal is often used. A thin layer (~2 nm) of silicon dioxide (SiO_2), a key element of the vast Si electronics industry because it is easy to form and produces an electrically stable self-interface with the low interface state density necessary for device operation, is placed between the ferromagnetic contact and Si to serve as a tunnel barrier. This facilitates spin injection by controlling the series resistance, and prevents intermixing between the ferromagnet and Si.

We probe the spin environment directly under the ferromagnet/SiO_2 contact using the three-terminal geometry shown in Fig. 4. In this geometry, a spin-polarized current is injected from contact 2 to 1, and a voltage is measured across contacts 2 and 3. Electrical spin injection produces spin accumulation in the transport channel under magnetic contact 2 and a corresponding output voltage. When a magnetic field B_Z is applied, the injected spins precess and dephase, and the spin accumulation decreases to zero. This Hanle effect is conclusive evidence of spin accumulation, and allows a direct measure of the spin lifetime and, subsequently, spin diffusion length, critical parameters for device operation. As shown in Fig. 5, we clearly observe Hanle precession of the electron spin accumulation in the Si channel under the contact over a wide range of temperatures up to 500 K.

Spin Lifetimes and Diffusion Lengths: The lineshape of the three-terminal Hanle data can be fit to ob-